PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

LEE, Han-Young
8th Fl., Seowon Bldg.
1675-1 Seocho-dong, Seocho-gu
Seoul 137-070
RÉPUBLIQUE DE CORÉE

Date of mailing (day/month/year) 10 April 2001 (10.04.01)	
Applicant's or agent's file reference P0052-KIOM	**IMPORTANT NOTIFICATION**
International application No. PCT/KR01/00368	International filing date (day/month/year) 09 March 2001 (09.03.01)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 14 August 2000 (14.08.00)
Applicant KIM, Chung-Sook et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
14 Augu 2000 (14.08.00)	2000/46916	KR	30 Marc 2001 (30.03.01)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

HA Ki-Nam

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

LEE, Han-Young
8th Fl., Seowon Bldg.
1675-1 Seocho-dong, Seocho-gu
Seoul 137-070
RÉPUBLIQUE DE CORÉE

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
10 April 2001 (10.04.01)	
Applicant's or agent's file reference	**International application No.**
P0052-KIOM	PCT/KR01/00368

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

KIM, Chung-Sook (all designated States)
HA, Hye-Kyung et al (for US)

International filing date	:	09 March 2001 (09.03.01)
Priority date(s) claimed	:	14 August 2000 (14.08.00)
Date of receipt of the record copy by the International Bureau	:	30 March 2001 (30.03.01)
List of designated Offices	:	

AP :GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
National :AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EE, ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA, MD,MG,MK,MN,MW,MX,MZ,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US, UZ,VN,YU,ZA,ZW

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [ ] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

HA Ki-Nam

Telephone No. (41-22) 338.83.38

ANNEX TO FORM PCT/IB/301

International application No.
PCT/KR01/00368

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P0052-KIOM		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/KR01/00368	09 MARCH 2001 (09.03.2001)	14 AUGUST 2000 (14.08.2000)
Applicant		
KIM, Chung-Sook et al		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ the statement that the subsequenlty furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawing** to be published with the abstract is Figure No. _____
   ☐ as suggested by the applicant.
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.
   ☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/00368

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/353

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC7: C07D; A61K

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the internationl search (name of data base and, where practicable, search trerms used)
CASLINK; ESPACENET

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 63-156720 A (KISSEI CO.) 29. 06. 88, see the whole document, (Family; none)	1-15
A	WO 95/03293 A (CHINOIN LTD.) 02. 02. 95, see the whole document	1-15
A	JP 60-048924 A (TAKETA LTD.) 16. 03. 85, see the whole document	1-15
A	US 6,040,333 A (SHERRY D.) 21. 03.00, see the whole document, (Family; none)	1-15
A	Fiorelli, G. et al., "Estrogen synthesis in human colon cancer epithelial cells", In; J. Steroid Biochem. Mol. Biol., 1999, 71(5-6), 223-230.	1-15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 JUNE 2001 (27.06.2001)	29 JUNE 2001 (29.06.2001)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	LEE, Yu Hyung Telephone No. 82-42-481-5603

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR01/00368

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 95/03293 A	02. 02. 95	AU 7236794 A	20. 02. 95
		CA 2167597 A	02. 02. 95
		CN 1129445 A	21. 08. 96
		EP 710234 A	08. 05. 96
		HU 68558 A	28. 06. 95
JP 60-048924 A	16. 03. 85	DE 3430799 A	14. 03. 85
		EP 135172 A	27. 03. 85
		IT 1179067 A	16. 09. 87

PCT REQUEST

P0052-KIOM

Original (for SUBMISSION) - printed on 09.03.2001 09:52:16 AM

0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	

0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.91 (updated 01.01.2001)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Korean Industrial Property Office (RO/KR)
0-7	Applicant's or agent's file reference	P0052-KIOM
I	Title of invention	A THERAPEUTIC AGENT OF OSTEOPOROSIS COMPRISING AN ACTIVE INGREDIENT OF QUERCETIN DERIVATIVES
II	Applicant	
II-1	This person is:	applicant and inventor
II-2	Applicant for	all designated States
II-4	Name (LAST, First)	KIM, Chung-Sook
II-5	Address:	4th Fl., Cheongam Bldg. 129-11 Cheongdam-dong, Kangnam-gu 135-100 Seoul Republic of Korea
II-6	State of nationality	KR
II-7	State of residence	KR
II-8	Telephone No.	82-2-3442-1994
II-9	Facsimile No.	82-2-3442-0220
II-10	e-mail	cskim@kiom.re.kr
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	HA, Hye-Kyung
III-1-5	Address:	1-408 Seoul Garden Apartment Dobong 1-dong, Dobong-gu 132-017 Seoul Republic of Korea
III-1-6	State of nationality	KR
III-1-7	State of residence	KR

PCT REQUEST

P0052-KIOM

Original (for SUBMISSION) - printed on 09.03.2001 09:52:16 AM

III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	SONG, Kye-Yong
III-2-5	Address:	922-6 Bangbae-dong, Seocho-gu 137-061 Seoul Republic of Korea
III-2-6	State of nationality	KR
III-2-7	State of residence	KR
IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	LEE, Han-Young
IV-1-2	Address:	8th Fl., Seowon Bldg. 1675-1 Seocho-dong, Seocho-gu 137-070 Seoul Republic of Korea
IV-1-3	Telephone No.	82-2-596-7200
IV-1-4	Facsimile No.	82-2-596-7280
IV-1-5	e-mail	LeePat@hitel.net
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST

P0052-KIOM

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V-5	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	Exclusion(s) from precautionary designations	NONE	
VI-1	Priority claim of earlier national application		
VI-1-1	Filing date	14 August 2000 (14.08.2000)	
VI-1-2	Number	2000-46916	
VI-1-3	Country	KR	
VI-2	Priority document request The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1	
VII-1	International Searching Authority Chosen	Korean Industrial Property Office (KIPO) (ISA/KR)	
VIII	Check list	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	26	-
VIII-3	Claims	7	-
VIII-4	Abstract	1	EZABST00.TXT
VIII-5	Drawings	0	-
VIII-7	TOTAL	38	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	Figure of the drawings which should accompany the abstract		
VIII-19	Language of filing of the international application	Korean	
IX-1	Signature of applicant or agent		
IX-1-1	Name (LAST, First)	LEE, Han-Young	

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	

PCT REQUEST

P0052-KIOM

Original (for SUBMISSION) - printed on 09.03.2001 09:52:16 AM

10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/KR
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

PCT (ANNEX - FEE CALCULATION SHEET)

P0052-KIOM

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(This sheet is not part of and does not count as a sheet of the international application)

0	For receiving Office use only	
0-1	International Application No.	
0-2	Date stamp of the receiving Office	

0-4	Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet				
0-4-1	Prepared using		PCT-EASY Version 2.91 (updated 01.01.2001)		
0-9	Applicant's or agent's file reference		P0052-KIOM		
2	Applicant		KIM, Chung-Sook, et al.		
12	Calculation of prescribed fees		fee amount/multiplier	total amounts (KRW)	
12-1	Transmittal fee	T	⇨	45,000	
12-2	Search fee	S	⇨	150,000	
12-3	International fee Basic fee (first 30 sheets)	b1	425,800		
12-4	Remaining sheets		8		
12-5	Additional amount	(X)	9,800		
12-6	Total additional amount	b2	78,400		
12-7	b1 + b2 =	B	504,200		
12-8	Designation fees Number of designations contained in international application		87		
12-9	Number of designation fees payable (maximum 6)		6		
12-10	Amount of designation fee	(X)	91,700		
12-11	Total designation fees	D	550,200		
12-12	PCT-EASY fee reduction	R	-131,000		
12-13	Total International fee (B+D-R)	I	⇨	923,400	
12-14	Fee for priority document Number of priority documents requested		1		
12-15	Fee per document	(X)	0		
12-16	Total priority document fee	P	⇨	0	
12-17	TOTAL FEES PAYABLE (T+S+I+P)		⇨	1,118,400	
12-19	Mode of payment		cash		

VALIDATION LOG AND REMARKS

13-2-1	Validation messages Request	Green? A translation of the international application into English will have to be prepared under the responsibility of the ISA selected.

PCT (ANNEX - FEE CALCULATION SHEET) P0052-KIOM

Original (for SUBMISSION) - printed on 09.03.2001 09:52:16 AM

		Green? Please note that the entire request (including the title of invention) must be in English
13-2-6	Validation messages Contents	Green? The international application contains no drawings. Please verify.
13-2-10	Validation messages For receiving Office/International Bureau use only	Green? Verify electronic data for consistency against printed form.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 March 2002 (07.03.2002)

PCT

(10) International Publication Number
WO 02/17909 A1

(51) International Patent Classification[7]: A61K 31/353

(21) International Application Number: PCT/KR01/00368

(22) International Filing Date: 9 March 2001 (09.03.2001)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
2000/46916 14 August 2000 (14.08.2000) KR

(71) Applicant *(for all designated States except US)*: **KOREA INSTITUTE OF ORIENTAL MEDICINE** [KR/KR]; 4th Fl., Seshin Bldg., 129-11 Cheongdam-dong,, Kangnam-gu, Seoul 135-765 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KIM, Chung-Sook** [KR/KR]; 4th Fl., Cheongam Bldg., 129-11 Cheongdam-dong, Kangnam-gu, 135-100 Seoul (KR). **HA, Hye-Kyung** [KR/KR]; 1-408 Seoul Garden Apartment, Dobong 1-dong, Dobong-gu, Seoul 132-017 (KR). **SONG, Kye-Yong** [KR/KR]; 922-6 Bangbae-dong, Seocho-gu, Seoul 137-061 (KR).

(74) Agent: **LEE, Han-Young**; 8th Fl., Seowon Bldg., 1675-1 Seocho-dong, Seocho-gu, Seoul 137-070 (KR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/17909 A1

(54) Title: A THERAPEUTIC AGENT OF OSTEOPOROSIS COMPRISING AN ACTIVE INGREDIENT OF QUERCETIN DERIVATIVES

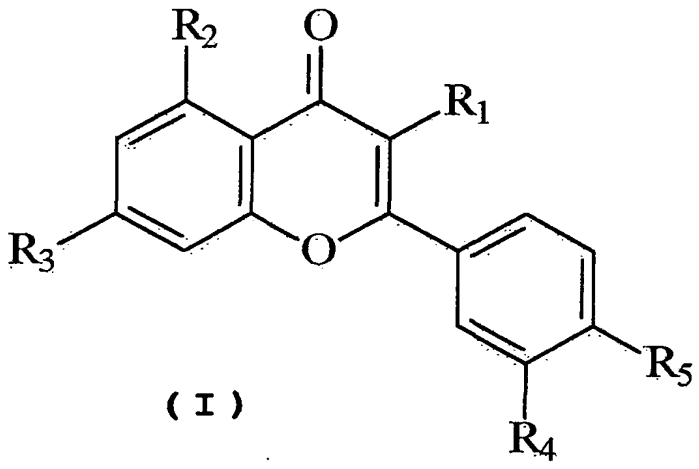
(57) Abstract: The present invention relates to a therapeutic agent of osteoporosis which comprises an active ingredient of quercetin derivatives. The quercetin derivatives of the invention can be practically applied for the treatment and prevention of osteoporosis, since they effectively inhibit osteoclast proliferation and stimulate osteoblast proliferation more than prior art therapeutic agents of osteoporosis, and increase trabecular bone area highly without changing hormone level in body and untoward effects on hematopoietic function and immune system.

A THERAPEUTIC AGENT OF OSTEOPOROSIS COMPRISING AN ACTIVE INGREDIENT OF QUERCETIN DERIVATIVES

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a therapeutic agent for osteoporosis which comprises an active ingredient of quercetin derivatives, more specifically, to a therapeutic agent for osteoporosis comprising an active ingredient of quercetin derivatives represented by the following general formula(I) which effectively stimulate osteoblast proliferation and inhibit osteoclast proliferation.



(I)

Description of the Prior Art

Osteoporosis is a disease characterized by the decrease of bone mass caused by mineral loss and the subsequent expansion of marrow cavity. Bones become brittle with the progress of the disease, and may be easily

fractured by a weak impact. Bone mass is affected by various factors such as genetic factors, nutritive condition, changes of hormone level, exercise and life style, and osteoporosis is known to be caused by aging, lack of exercise, low body weight, smoking, low calcium diet, menopause, and ovariectomy. In women, decrease of bone mass begins at the age of 30, and around menopause, concentration of estrogen rapidly decreases and vast amount of B-lymphocytes are accumulated by the similar mechanism to that of B-lymphocyte accumulation by IL-7(interleukin-7), and subsequent pre-B cell accumulation results in increased level of IL-6 which activates osteoclasts, thus, bone mass becomes decreased. In aged people, especially in women of postmenopause, osteoporosis is not the avoidable disease although the severity of the symptom may vary, therefore, many research groups and pharmaceutical companies have made a great deal of efforts for development of therapeutic agents for bone diseases to prevent and treat osteoporosis upon an increase of elderly population.

Therapeutic agents for osteoporosis now being used include estrogen preparations, androgenic anabolic steroid preparations, calcium supplements, phosphate preparations, fluoride preparations, ipriflavone, vitamin D3, etc. In recent years, novel drugs for osteoporosis have been developed, which include Aminobisphosphonate by Merck Co.(U.S.A.) in 1995 and Raloxifene which plays a role of selective estrogen receptor modulator(SERM) by Eli Lilly Co.(U.S.A.) in 1997.

Therapeutic agents for osteoporosis mentioned above are mostly estrogen substances which are known to cause adverse side effects such as cancer, cholelithiasis, and thrombosis. Since long term administration of drug is inevitable in the treatment of osteoporosis, there is a continuing need to develop novel effective agents which can replace estrogen with high safety even when administered for a prolonged period of time.

As estrogen substitutes, phytoestrogens such as soybean isoflavone have been reported. Phytoestrogen, first reported in 1946, was found interim of verifying the cause of clover disease which was named for the high increase(over 30%) of infertility of the sheep fed with red clover(*Trifolium subterraneum* var. Dwalganup). The cause of clover disease turned out to be an estrogen-like isoflavonoid contained in the plant, hence, the compound obtained from the plant has been named 'phytoestrogen'. After that, compounds reported as phytoestrogen includes isoflavone compounds such as daidzein, genistein, formononetin, and biochanin A, coumestan compounds such as coumestrol, lignan compounds such as enterolactone, and phenol compounds such as enterodiol. Such phytoestrogens exist mostly in the form of aglycone, 6'-O-acetylglucoside or 6'-O-malonylglucoside, and daidzein and genistein exist in the form of 7-O-glucoside. Among aforementioned compounds, glucosides are known to be hydrolysed with enterobacteria or gastric acid and absorbed in the form of aglycone which is a free isoflavone. The researches have revealed that the said phytoestrogens function similarly to the animal estrogens. That is, the phytoestrogen inhibit proliferation of breast cancer cells by binding to the estrogen receptor and have been reported to be used as the estrogen substitute for the treatment of cardiovascular diseases and other symptoms occurring in the postmenopause women. However, the said phytoestrogens are not widely used for the treatment and prevention of osteoporosis due to the insufficient pharmaceutical effectiveness and high cost required for the isolation and purification from natural products.

Under the circumstances, are strong reasons for developing and exploring alternative compounds with safety and effectiveness for the treatment and prevention of osteoporosis, which can be prepared in an economical manner.

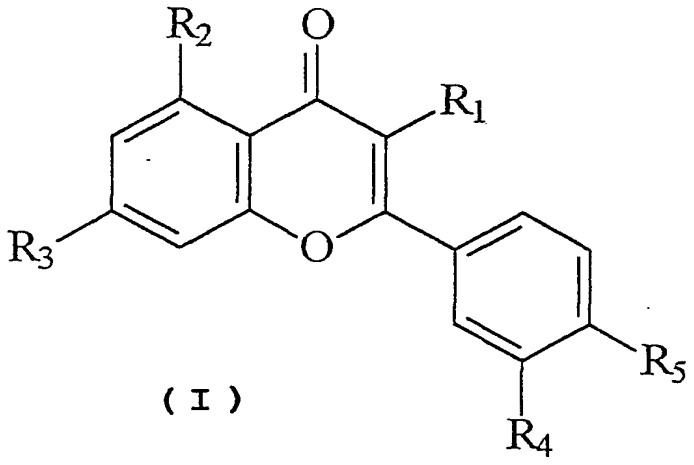
SUMMARY OF THE INVENTION

The present inventors have made an effort to develop an effective substitute agent for the treatment and prevention of osteoporosis, which is safe and economical, and have found that chemically synthesized quercetin derivatives have activities of stimulating osteoblast proliferation and inhibiting osteoclast proliferation, without any adverse side effects on internal organs, thus, quercetin derivative can be employed as an active ingredient of a therapeutic agent for osteoporosis.

A primary object of the present invention is, therefore, to provide a therapeutic agent for osteoporosis which comprises an active ingredient of quercetin derivatives.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a therapeutic agent for osteoporosis which comprises an active ingredient of quercetin derivatives represented by the following general formula(I) and pharmaceutically acceptable carriers:



(I)

wherein,

R_1 is gentiotriose, glucopyranose, O-arabinofuranose, O-diglucopyranose, O-galactopyranose, O-galactoside-gallate, O-gentiobiose, O-glucopyranose, O-glucuronide, O-neohesperidose, O-rhamnopyranose, O-rutinose, O-sophorose, O-xylopyranose, OCH_3, OH, rhamnogentiobiose, rhamnoglucose or sulfate;

R_2 is OH or O-glucopyranose;

R_3 is OCH_3, OH, O-glucopyranose, O-glucuronopyranose or glucopyranose;

R_4 is OCH_3 or OH; and,

R_5 is OCH_3, OH, O-glucopyranose or O-glucose.

Among the quercetin derivatives represented by general formula(I), well-known compounds are classified as follows: (i) a derivative group of the formula I wherein R_2 to R_5 are OH and R_1 varies, includes quercetin where R_1 is OH, avicularoside where R_1 is O-α-L-arabinofuranose, guiajaverin where R_1 is O-arabinopyranose, hyperoside where R_1 is O-β-D-galactopyranose, isohyperoside where R_1 is O-β-D-galactopyranose, isoquercitrin where R_1 is O-glucopyranose, multinoside A where R_1 is O-[β-D-glucopyranosyl-(1-4)-α-L-rhamnopyranose], multinoside A acetate where R_1 is (6-O-acetyl)-β-D-glucopyranosyl-(1-4)-α-L-rhamnopyranose, quercitrin where R_1 is O-α-L-rhamnopyranose, rutin where R_1 is O-β-D-rutinose, quercetin-3-O-(2"-O-β-D-glucopyranosyl)-α-L-rhamnopyranoside where R_1 is O-(2"-O-β-D-glucopyranosyl)-α-L-rhamnopyranose, quercetin-3-O-(6"-O-galloyl)-glucopyranoside where R_1 is O-(6"-O-galloyl)-glucopyranose, quercetin-3-O-(6'"-O-p-coumaroyl-β-D-glucopyranosyl-(1-2)-α-L-rhamnopyranoside) where R_1 is O-(6'"-O-p-coumaroyl-β-D-glucopyranosyl-(1-2)-α-L-rhamnopyranose, quercetin-3-O-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl-(1-4)-α-L-rhamnopyranoside where R_1 is O-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl-(1-4)-α-L-rhamnopyranose, quercetin-3-O-[2"-O-6'"-O-p-(7""-O-β-D-glucopyranosyl)coumaroyl-β-D-glucopyranosyl]-α-L-rhamnopyranoside where R_1 is O-[2"-O-

6''''-O-p-(7''''-O-β-D-glucopyranosyl)coumaroyl-β-D-glucopyranosyl]-α-L-rhamnopyranose, quercetin-3-O-[6''''-p-coumaroyl-β-D-glucopyranosyl-β-(1-4)-rhamnopyranoside] where R_1 is O-[6''''-p-coumaroyl-β-D-glucopyranosyl-β-(1-4)-rhamnopyranose], quercetin-3-O-[α-L-rhamnopyranosyl(1-2)-α-L-rhamnopyranosyl -(1-6)-β-D-glucopyranoside] where R_1 is O-[α-L-rhamnopyranosyl(1-2)-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranose], quercetin-3-O-[α-rhamnopyranosyl(1-4)α-L-rhamnopyranosyl(1-6)β-D-galactopyranoside] where R_1 is O-[α-rhamnopyranosyl(1-4)α-L-rhamnopyranosyl(1-6)β-D-galactopyranose], quercetin-3-O-[α-rhamnopyranosyl-(1-2)]-[β-glucopyranosyl-(1-6)]-β-D-galactopyranoside where R_1 is O-[α-rhamnopyranosyl-(1-2)]-[β-glucopyranosyl-(1-6)]-β-D-galactopyranose, quercetin-3-O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-galactopyranoside] where R_1 is O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-galactopyranose], quercetin-3-O-α-L-rhamnopyranosyl-(1-2)-β-D-galactopyranoside where R_1 is O-α-L-rhamnopyranosyl-(1-2)-β-D-galactopyranose, quercetin-3-O-β-D-diglucopyranoside where R_1 is O-β-D-diglucopyranose, quercetin-3-O-β-D-galactoside-2"-gallate where R_1 is O-β-D-galactoside-2"-gallate, quercetin-3-O-β-D-glucopyranoside -(1-6)-β-D-galactopyranoside where R_1 is O-β-D-glucopyranoside-(1-6)-β-D-galactopyranose, quercetin-3-O-β-D-glucopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-6)-β-D-galactopyranoside where R_1 is O-β-D-glucopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-6)-β-D-galactopyranose, quercetin-3-O-β-D-glucuronide where R_1 is O-β-D-glucuronide, quercetin-3-O-β-D-xylopyranoside where R_1 is O-β-D-xylopyranose, quercetin-3-O-diglucospyranoside where R_1 is O-diglucospyranose, quercetin-3-O-gentiobioside where R_1 is O-gentiobiose, quercetin-3-O-glucopyranosylgalactopyranoside where R_1 is O-glucopyranosylgalactopyranose, quercetin-3-O-neohesperidoside where R_1 is O-neohesperidose, quercetin-3-O-sophoroside where R_1 is O-sophorose, quercetin-3-gentiotrioside where R_1 is gentiotriose, quercetin-3-methyl ether where R_1 is OCH_3, quercetin-3-rhamnogentiobioside

where R_1 is rhamnogentiobiose, quercetin-3-rhamnoglucoside where R_1 is rhamnoglucose, and quercetin-3-sulfate where R_1 is sulfate; (ii) a derivative group of the formula I wherein R_1 is -OH, three functional groups out of R_2 to R_5 are -OH, and the rest one functional group varies, includes isorhamnetin where R_4 is OCH_3, quercimeritrin where R_3 is O-β-D-glucopyranose, rhamnetin where R_3 is OCH_3, quercetin-5-O-β-D-glucopyranoside where R_2 is O-β-D-glucopyranose, quercetin-7-O-β-D-glucuronopyranoside where R_3 is O-β-D-glucuronopyranose, and spireaoside where R_5 is O-glucose; (iii) a derivative group of the formula I wherein three functional groups out of R_1 to R_5 are OH and the rest two functional groups vary, includes rhamnazin where R_3 and R_4 are OCH_3, quercetin-3',4'-di-methyl ether where R_4 and R_5 are OCH_3, quercetin-3,3'-dimethyl ether where R_1 and R_4 are OCH_3, quercetin-3,7-dimethyl ether where R_1 and R_3 are OCH_3, quercetin-3-O-[2"-O-(6'"-O-p-coumaroyl)-β-D-glucopyranosyl]-α-L-rhamnopyranosyl-7-O-β-D-glucopyranoside where R_1 is O-[2"-O-(6'"-O-p-coumaroyl)-β-D-glucopyranosyl]-α-L-rhamnopyranose and R_3 is O-β-D-glucopyranose, quercetin-3-O-[2"-O-6'"-O-p-(7""-O-β-D-glucopyranosyl)coumaroyl-β-D-glucopyranosyl]-α-L-rhamnopyranoside-7-O-β-D-glucopyranoside where R_1 is O-[2"-O-6'"-O-p-(7""-O-β-D-glucopyranosyl)coumaroyl-β-D-glucopyranosyl]-α-L-rhamnopyranose and R_3 is O-β-D-glucopyranose, quercetin-3-O-rutinoside-7-O-β-D-glucopyranoside where R_1 is O-rutinose and R_3 is O-β-D-glucopyranose, quercetin-3-O-α-L-arabinopyranosyl-7-O-β-D-glucopyranoside where R_1 is O-α-L-arabinopyranosyl and R_3 is O-β-D-glucopyranose, quercetin-7-O-β-D-glucopyranoside-3-O-sophoroside where R_1 is O-sophorose and R_3 is O-β-D-glucopyranose, quercetin-3-O-galactopyranosyl-7-O-diglucopyranoside where R_1 is O-galactopyranose and R_3 is O-glucopyranose, quercetin-3-O-glucopyranosyl-7-diglucopyranoside where R_1 is O-glucopyranose and R_3 is O-glucopyranose, quercetin-3,7-diglucopyranoside where R_1 is glucopyranose and R_3 is glucopyranose, quercetin-3-gentiobiosyl-7-glucopyranoside

where R_1 is gentiobiose and R_3 is glucopyranose, and quercetin-3,4'-di-O-β-D-glucopyranoside where R_1 and R_5 are O-β-D-glucopyranose; and (iv) a derivative group of the formula I wherein more than three functional groups vary, includes quercetin-3,4',7-trimethyl ether where R_1, R_3 and R_5 are OCH_3, and R_2 and R_4 are OH, and quercetin-3,3',4',7-tetramethyl ether where R_1, R_3, R_4 and R_5 are OCH_3, and R_2 is OH.

Quercetin having same OH groups in R_1 to R_5 of the above general formula(I) is a phenolic compound found in over 4000 kinds of plants in nature and is known as one of the phytoestrogens. It has a molecular formula of $C_{15}H_{10}O_7$ with resonance structures and a molecular weight of 302.33 g/mole and also known as vitamin P following the chemical structure identification in 1936. Quercetin is a rutin, a glycoside wherein sugar is linked via β-linkage and widely distributed in plants such as clover flower, pollen of common ragweed, and shell and stem of various plants, as well as in onion, kale, broccoli, lettuce, tomato, and apple. Quercetin has been verified not only to play an important role in maintenance of capillary wall integrity and capillary resistance(see: Gabor et al., *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medical Properties*, 280: 1-15, 1988; Havsteen et al., *Biochemical Pharmacology*, 32:1141-1148, 1983) but also to have antioxidation activity, vitamin P activity, ultraviolet absorbing activity, antihypertensive activity, antiarrhythmic activity, antiinflamatory activity, antiallergic activity, anticholesteremic activity, suppressive activity on liver toxicity, and therapeutic effect on infertility, thus, it may be expected to use quercetin widely in foods, medical and pharmaceutical products, and cosmetics. However, there has been no report on the use of quercetin for prevention and treatment of osteoporosis.

The therapeutic agent for osteoporosis of the invention comprising an active ingredient of quercetin derivative is illustrated below.

In order to search for the effects of quercetin derivatives on proliferation of osteoblasts and osteoclasts, the present inventors compared the effect of quercetin with that of phytoestrogen genistein which is known to be an effective agent for treatment of osteoporosis, and have found that quercetin has superior effects to genistein for activation of osteoblast proliferation, increase of alkaline phosphatase activity, and inhibition of osteoclast proliferation.

Furthermore, in ovariectomized rats, administration of quercetin derivatives has been found not to bring about changes in hormone level, proving that quercetin is a safe agent not causing uterine hypertrophy, an adverse side effect of estradiol which is being used as a therapeutic agent for osteoporosis currently. Also, quercetin derivatives were shown to be more effective than estradiol on increase of trabecular bone area of tibia which is apt to drastic change in trabecular bone area, and to have no adverse effect on hematopoietic function and immune system.

Therefore, quercetin derivatives of the invention, based on above results, have been found not only to have superior effects to currently using phytoestrogen genistein for activation of osteoblast proliferation and inhibition of osteoclast proliferation but also to have little side effects, bring about little change in hormone level and have no adverse effect on hematopoietic function and immune system, substantiating the use of quercetin derivatives as a therapeutic or preventive agent for osteoporosis.

Formulation

The said quercetin derivatives having superior effect on treatment of osteoporosis may be mixed with pharmaceutically acceptable excipients including binders such as polyvinylpyrrolidone, hydroxypropylcellulose, etc., disintegrating agents such as calcium carboxymethylcellulose, sodium glycolate starch, etc., diluting agents such as corn starch, lactose, soybean oil, crystalline cellulose, mannitol, etc., lubricating agents such as magnesium stearate, talc, etc., sweeteners such as sucrose, fructose, sorbitol, aspartame, etc., stabilizing agents such as sodium carboxymethylcellulose, α- or β-cyclodextrin, vitamin C, citric acid, white wax, etc, preservatives such as paraoxymethylbenzoate, paraoxypropylbenzoate, sodium benzoate, etc., and aromatics such as ethylvanillin, masking flavor, flavonomenthol, herb flavor, etc. to prepare pharmaceutical formulations for oral or parenteral administration such as tablets, capsules, soft capsules, liquids, ointments, pills, powders, suspensions, emulsions, syrups, suppositories or injections. Also, to augment efficacy of prevention and treatment of osteoporosis, calcium or vitamin D_3 may be added to the formulations. For parenteral administration of the pharmaceutical preparation of the invention, subcutaneous, intravenous, intramuscular or intraperitoneal injection may be employed. For parenteral administration, quercetin derivative may be mixed with stabilizer or buffer in water to prepare solution or suspension which can be produced as single-dose formulations of ampule or vial.

Dosage

The effective amount of quercetin in the therapeutic agent for osteoporosis of the invention is 2 to 20mg/kg, preferably 8 to 12mg/kg, which may be administered to the patient more than once a day depending on the patient's age, gender, degree of seriousness, way of administration, or purpose of prevention.

Safety

The toxicity of the quercetin derivatives of the invention has been reported in the literature(see: M. Sullivan *et al., Proc. Soc. Exp. Biol. Med.,* 77:269, 1951) for the cases of oral administration and intraperitoneal administration to the mice, and LD_{50} of orally administered quercetin was not less than 160mg/kg, approving that quercetin is safe. In the present invention, liver, kidney, brain, uterus, skin and tibia were examined for the side effect of quercetin, which revealed that the weight of liver, kidney, brain, skin and tibia was not affected, moreover, uterine hypertrophy, a side effect of currently used therapeutic agents, was not observed with quercetin, proving that quercetin derivative as a hormone preparation can be used safely as a therapeutic agent for osteoporosis.

The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention.

Example 1: Effect of quercetin on osteoblast proliferation

To analyse the effect of quercetin on osteoblast proliferation, human osteoblast-like cell line Saos-2 was employed and a phytoestrogen genistein was employed as a comparative agent which has been intensively studied as a therapeutic agent for osteoporosis.

Example 1-1: Selection and culture of osteoblasts

Saos-2 cell line which has similar properties to osteoblasts was obtained from Korean Cell Line Bank affiliated to the Cancer Research Institute of School of Medicine, Seoul National University.

Saos-2 cells were seeded in a RPMI 1640 medium(Gibco BRL, U.S.A.) supplemented with 10%(v/v) FBS, 100unit/ml penicillin, 100 μg/ml streptomycin and grown to form a monolayer in an incubator at 37°C under an environment of 5%(v/v) CO_2 and saturated humidity. The culture was fed with fresh medium 2 to 3 times a week and subcultured once a week using 0.25%(w/v) trypsin.

Example 1-2: Cell proliferation depending on concentrations of the agents

Saos-2 cells were distributed into a 96-well plate(20,000 cells/well) and quercetin in 1% DMSO was added to a final concentration of 10^{-2} to 10^{-9}mg/ml, 6 wells per each concentration. As a control group, cells without quercetin were used, and as a comparative group, the cells treated with various concentrations of genistein, being studied as a therapeutic agent for osteoporosis, were used. Cells were grown in an incubator at 37°C for 3 days and incubated 4 more hours under the same condition after adding MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Triazolyl Blue) to a concentration of 0.05mg/ml. Then, purple colored formazan formed in proportion to the number of viable cells was dissolved in DMSO and measured OD at 550nm employing ELISA reader.

Cell proliferation rate(%) was evaluated by calculating the ratio of the OD of quercetin added well to the OD of control well, wherein, average value of ODs from 6 wells treated with the same concentration of quercetin was employed(see: Table 1).

cell proliferation rate(%)= {(average value of OD at 550nm of quercetin-treated wells - average value of OD at 550nm

of empty wells)/average value of OD at 550nm of control wells}X100

Example 1-3: Analysis of alkaline phosphatase(ALP) activity

Since osteoblasts have cell specific alkaline phosphatase activity, the effect of quercetin of the invention on ALP activity in osteoblasts was evaluated as follows: the number of cells, concentration of tested agent, and culture condition were same as those used in MTT experiment of Example 1-2, and cells were harvested after 3 day-incubation. Genistein was used as a comparative agent. ALP activity was evaluated by analysing changes of OD at 405nm result from hydrolysis of p-nitrophenylphosphate to p-nitrophenol and phosphate(see: Table 1).

Table 1: Effect of quercetin on osteoblast proliferation

Concentration (mg/ml)	Quercetin (% of control group)		Genistein (% of control group)	
	MTT assay	ALP activity	MTT assay	ALP activity
Control group	100.0 ±2.5	100.0 ±1.6	100.0 ±0.6	100.0 ±7.3
1×10^{-9}	93.1 ±0.8*	98.1 ±0.0	91.3 ±0.6*	106.1 ±6.4
1×10^{-8}	93.9 ±0.8	104.4 ±3.9	96.9 ±2.7	101.5 ±8.8
1×10^{-7}	98.6 ±1.0	101.2 ±3.1	95.9 ±1.6	109.3 ±9.6
1×10^{-6}	96.0 ±1.0	127.2 ±3.5**	90.5 ±0.9**	103.8 ±8.7
1×10^{-5}	95.8 ±1.1	116.5 ±3.7	97.3 ±1.6	113.5 ±7.3
1×10^{-4}	96.5 ±0.8	113.5 ±2.3	95.7 ±0.7	121.1 ±6.2
1×10^{-3}	98.3 ±0.8	107.3 ±1.5	85.5 ±1.1**	98.8 ±6.9
1×10^{-2}	108.6 ±2.2**	106.1 ±4.3	66.2 ±2.8**	62.3 ±3.4

*: $p<0.05$

**: $p<0.01$

As shown in Table 1 above, in the cell proliferation experiment using MTT method, the cells treated with various concentrations of quercetin in the range of $1x10^{-9}$ to $1x10^{-3}$ mg/ml did not show any difference from the control cells which were not treated with the agent, while quercetin showed maximum cell proliferation effect of 109% of control cell proliferation at a concentration of $1x10^{-2}$ mg/ml($p<0.01$). On the other hand, genistein, a comparative agent, showed 91%($p<0.05$) at a concentration of $1x10^{-9}$mg/ml, 90.5%($p<0.01$) at a concentration of $1x10^{-6}$mg/ml, 86%($p<0.01$) at a concentration of $1x10^{-3}$mg/ml, and 66%($p<0.01$) at a concentration of $1x10^{-2}$mg/ml, implying that genistein exert rather inhibitory effect than stimulatory effect on proliferation of osteoblasts.

In the experiment of assaying ALP activity, quercetin showed its maximum ALP activation effect of 127%($p<0.01$) of control ALP activity at a concentration of $1x10^{-6}$mg/ml, while genistein showed its maximum ALP activation activity of 121% at a concentration of $1x10^{-4}$mg/ml, indicating that the ALP activation effect of quercetin of the invention is about 100 fold higher than that of genistein. Therefore, quercetin of the invention is more effective on the stimulation of osteoblast proliferation and activation of ALP activity than genistein which is studied intensively as a therapeutic agent for osteoporosis in recent years.

Example 2: Effect of quercetin on osteoclast proliferation

To examine whether quercetin have inhibitory effect on the proliferation of osteoclasts, experiments were carried out as followings.

Example 2-1: Selection and culture of osteoclasts

ICR mice(Korea Research Institute of Chemical Technology, Taejon, Korea) were fed with calcium deficient diet(ICN Biomedicals, Inc., Ohio, U.S.A.) for 4 weeks to activate osteoclasts. The right and left tibiae and femurs of the calcium deficient rats were removed avoiding contamination of surrounding muscle tissues. Femurs and right and left tibiae, classified on the clean bench and kept on ice separately, were added into the α-MEM containing 100 μg/ml streptomycin and then vigorously shaken respectively to extract osteoclasts into the medium. After kept on ice for 5 minutes, the cell suspension was centrifuged at 800xg for 3 minutes and the cell pellet was resuspended in a α-MEM nutrient medium supplemented with 10% FBS, 100 μg/ml streptomycin and 100unit/ml penicillin. The cell suspension was distributed into wells of a 24-well plate at a cell number of $3.5x10^6$/well.

Example 2-2: Cell proliferation depending on concentrations of quercetin

To the osteoclasts obtained in Example 2-1 above, quercetin was added to yield concentrations of $1x10^{-8}$ to $1x10^{-2}$mg/ml. On day 2, the cells were subjected to tartrate-resistant acid phosphatase(TRAP) staining using a commercially available kit(Sigma Chemical Co., U.S.A.), followed by counting of osteoclasts which are TRAP-positive multinucleated cells(MNC), judged by more than three nuclei in a cell stained red(see: Table 2).

Table 2: Effect of quercetin on osteoclast proliferation

Concentration (mg/ml)	Number of osteoclast (% of control group)
Control group	100.0±8.1
1×10^{-8}	100.9±1.8
1×10^{-6}	96.8±2.7
1×10^{-4}	89.6±3.2

1×10^{-3}	61.1±4.1*
1×10^{-2}	24.7±5.7**

*: p<0.05,

**: p<0.01

As shown in Table 2 above, while quercetin at concentrations between $1x10^{-8}$ to $1x10^{-4}$mg/ml exerted little inhibitory effect on the osteoclast proliferation, the cell numbers at quercetin concentration of $1x10^{-3}$mg/ml and $1x10^{-2}$mg/ml was 61%(p<0.05%) and 25% of control cell number respectively, showing that quercetin exerted remarkable inhibitory effect on the osteoclast proliferation.

Based on the results of Examples 1 and 2, it was clearly demonstrated that quercetin is a potential therapeutic agent for osteoporosis which exerts stimulatory effect on osteoblast proliferation and inhibitory effect on osteoclast proliferation at a concentration of 10^{-2}mg/ml.

Example 3: Effect of quercetin on ovariectomized rats

Female SD(Sprague-Dawley) rats, a model animal for type I osteoporosis occurring after menopause were employed for evaluating pharmacological effectiveness of quercetin. Female rats(10 weeks old) weighing 200 to 300g, obtained from the Korea Research Institute of Chemical Technology were employed as experimental animals. Experiment was carried out by the procedure which comprises removing ovary, administration of agents to the each group of rats, and at certain days after ovariectomy, the rats were sacrificed and subjected to analyses including measurement of body weight, examination of internal organs, measurement of trabecular bone area, complete blood count, and biochemical analyses of plasma.

Example 3-1: Ovariectomy and administration of the agents

Rats of control group and test group, except Sham group(normal group), were overiectomized as follows: a female rat was systemic anesthetized by intramuscular injection with 5mg/100g Ketamin(Yuhan Corporation, Korea) and 1mg/100g Xylazine(Beyer Korea, Korea) to the femur muscle of left and right hind limbs, and then, fur of lower abdominal region was shaved, operation area was sterilized with Potadin liquid(Iodine, Samil Pharm. Co., Ltd., Korea) in lying position, about 2cm of abdominal skin, abdominal muscle, and peritoneum was cut in the middle under aseptic condition, ovary was exposed using sterilized forceps, followed by removal of both left and right ovaries after ligaturing of oviducts using silk threads. Subsequently, 0.3ml of antibiotics(Sulfaforte®-4, Yoonee Chemical Co., Ltd., Korea) was injected intraperitoneally to prevent infection, and then peritoneum, andominal muscle and skin were sutured with silk threads or nylon threads.

The Sham group, animals operated upon for the surgery as in the ovariectomized rats except for removing ovary, were employed to compare the changes caused solely by ovariectomy in control group which were ovariectomized but no agent was administered. Control group was employed to compare the changes caused by administration of agents in test group which were ovariectomized and administered with testing agents.

When test agents were administered, for a certain period of time before and after administration, 1.5ml of blood was sampled from tail vein using a catheter(B.D Co.: 24G) and subjected to complete blood count(Coulter Co.: JT) and biochemical analyses of plasma(Crone Co.: Airon® 200). During autopsy, blood was sampled from caudal venae cavae and subjected to the analyses above. And then, each sample was frozen to store for measurement of trabecular bone area of femur and examination of internal organs.

One week after operation, rats in Sham group and control group were intraperitoneally injected with 10% Tween 80 solution, the rats in E2 group were injected with 17β-estradiol at a concentration of 1 μg/kg/day, the rats in test group were injected with quercetin or genistein at a concentration of 10mg/kg/day for 9 weeks, and the rats in each group were subjected to body weight measurement once a week. During the period of administration, blood was sampled once a week. After 9-week administration, entire blood was withdrawn with heparin treatment. Following complete blood count(CBC), the blood was centrifuged at 3,000rpm for 20 minutes to obtain plasma which was stored at -70°C until use. For measurement of bone mineral density, the lumbar spine L5 and L6, and right tibia were removed and stored separately in 4%(v/v) formalin solution.

Example 3-2: Body weight change depending on quercetin administration

The body weight of the rats in Sham group, E2 group treated with 17β-estradiol and test group treated with quercetin or genistein respectively, was measured once a week for 10 weeks after operation(see: Table 3).

Table 3: Mesurement of body weight changes depending on drug administration

Time (week)	Weight(g)				
	Control group	Sham group	E2-treated group	Quercetin-treated group	Genistein-treated group
Before operation	219.39±4.05	220.70±4.63	228.51±8.11	221.87±7.57	217.55±7.24
1 after operation	244.98±3.00	231.51±4.68	249.50±8.16	241.73±4.83	242.12±5.96

2 after operation	274.29±3.68**	236.40±5.06##	264.97±8.35	271.70±5.79**	270.00±8.05**
3 after operation	299.37±3.74**	245.56±4.79*##	279.87±8.15**	295.00±3.89**	296.20±7.68**
4 after operation	315.20±3.84**	248.96±5.02*##	292.83±9.25**	312.07±5.95**	310.80±7.80**
5 after operation	320.30±4.83**	255.43±5.14**##	296.96±9.44**	320.25±6.76**	317.29±7.93**
6 after operation	329.03±5.05**	261.49±6.46**##	304.49±8.40**	326.68±6.73**	327.19±8.31**
7 after operation	337.39±5.93**	264.78±5.53**##	313.04±8.73**	333.25±7.61**	332.80±9.23**
8 after operation	340.01±6.60**	268.16±5.40**##	315.87±8.32**	335.09±6.65**	336.38±9.01**
9 after operation	347.96±7.58**	273.81±4.54**##	319.95±9.47**	343.02±6.96**	342.71±8.26**
10 after operation	356.73±7.13**	275.22±4.30**##	320.00±5.90**#	346.27±6.39**	347.23±7.57**

*: p<0.05, **: p<0.01, compared with before operation

#: p<0.05, ##: p<0.01, compared with control group

As shown in Table 3, body weight of Sham group began to increase 3 weeks($p<0.05$) after operation and that of control group began to increase 2 weeks($p<0.01$) after operation. That is, control group showed rapid increase of body weight compare to Sham group, and such increase of body weight was slowed down after administration of estradiol, and E2 group showed slower increase of body

weight compare to control group($p<0.05$) 20 weeks after operation. Meanwhile, the test group administered with phytoestrogen quercetin or genistein at a concentration of 10mg/kg/day respectively showed rapid increase of body weight even after removing ovary similar to control group. Thus, quercetin administration was found not to bring about meaningful changes in hormone level in the body.

Example 3-3: Changes in the weight of internal organ by quercetin

To find out quercetin effect on internal organ of test animal, liver, kidney, brain, uterus, skin, and tibia were removed from the test animals administered with test agents for 9 weeks after operation and wet weight of each organ was measured(see: Table 4).

Table 4: Changes in the weight of internal organ after drug administeration

	Control group	Sham group	E2-treated group	Quercetin-treated group	Genistein-treated group
Liver (g)	9.84±0.33	9.52±0.48	9.22±0.43	9.07±0.30	10.03±0.36
Kidney (g)	1.95±0.09	1.91±0.05	1.85±0.09	1.84±0.05	1.83±0.03
Brain (g)	2.03±0.04	1.93±0.02	1.98±0.05	1.98±0.04	1.98±0.03
Tibia (g)	0.559±0.025	0.514±0.013	0.504±0.019	0.554±0.019	0.537±0.008
Skin (mg)	193±7	169±8	193±6	197±11	188±9
Uterus (mg)	79±4	450±29**	279±10**	85±6	106±3

**: $p<0.01$

As shown in Table 4, in case of the weight of liver, kidney, brain, tibia, and skin, normal Sham group, ovariectomized control group and test group did not show

differences among groups. However, in case of weight of uterus which is affected by the estrogen secreted from ovary, ovariectomized control group showed significant decrease($p<0.01$) compare to Sham group, and administration of E2 after removing ovary suppressed atrophy of uterus($p<0.01$) compare to control group. Administration of phytoestrogen quercetin or genistein did not give rise to change in weight of uterus, on the other hand, E2 which is a currently used therapeutic agent for osteoporosis showed side effect such as uterine hypertrophy, showing that quercetin can be used safely as a therapeutic agent for osteoporosis without adverse side effect.

Example 3-4: Changes in the trabecular bone area by quercetin

Trabecular bone area(TBA) of lumbar and tibia removed from the rats of each group which was treated with various agents for 9 weeks were measured as follows: that is, using a digitalizer of quantitative image analysis system(Wild Leitz Co.), image of each trabecula was obtained on computer monitor by drawing a contour of the trabecula, and then, using a computer, calculated were average areas of trabeculae within a rectangle of $2x10^6$ μm^2 area wherein the width is about 2/3 of the length of growth plate which located underneath of growth plate at proximity of tibia. Also, following the number of trabeculae within the rectangle were obtained, average area was multiplied by the number of trabeculae to obtain trabecular bone area of each sample bone, which was analyzed statistically(see: Table 5).

Table 5: Changes in the trabecular bone area of tibia depending on drug administration

	TBA ($\times 10^4$ μm^2)	Change Rate(%)
Control group	34.62 ±2.62	100.00 ±7.55

Sham group	85.55 ±5.31**	247.07 ±15.33**
E2-treated group	51.40 ±2.28	148.46 ±6.59
Quercetin-treated group	55.52 ±7.68*	160.34 ±22.17*
Genistein-treated group	47.65 ±2.07	137.62 ±5.98

*: $p<0.05$,

**: $p<0.01$

As shown in Table 5, in case of tibia, the TBA of control group was 34.62×10^4 μm^2 which is a significantly decreased value compare to normal Sham group of 85.55×10^4 μm^2 ($p<0.01$), showing that osteoporosis have occurred in control group, and such decreased TBA was increased again by treatment with E2, quercetin or genistein to 148%, 160%, and 138% of TBA of control group respectively, especially in case of quercetin, remarkable increase of TBA was monitored($p<0.05$).

TBAs of lumbars removed from the animal treated with test agents for 9 weeks were measured employing the same method above(see: Table 6).

Table 6: Changes in the trabecular bone area of lumbars depending on drug administration

	TBA ($\times 10^4$ μ m^2)	Change Rate(%)
Control group	67.53 ±2.31	100.00±3.42
Sham group	93.70 ±5.29**	138.76±7.84**
E2-treated group	89.16 ±2.83**	132.04±4.19**
Quercetin-treated group	87.38 ±4.53*	129.40±6.71*
Genistein-treated group	86.58 ±3.00*	128.23±4.45*

*: $p<0.05$,

**: $p<0.01$

As shown in Table 6, in case of lumbar, the TBA of control group was $67.53 \times 10^4 \mu m^2$ which is a decreased value compare to Sham group of $93.70 \times 10^4 \mu m^2$ ($p<0.01$), but, such decreased TBA was increased again by treatment with E2, quercetin or genistein to 132%($p<0.01$), 129%($p<0.05$) and 128%($p<0.05$) of TBA of control group respectively, showing that these test agents exerted suppressing effect on decrease of TBA caused by ovariectomy. Especially, quercetin showed more significant increase of TBA in tibia which is apt to drastic change in TBA than E2 a currently used therapeutic agent for osteoporosis, showing that quercetin is a more effective therapeutic agent not causing uterine hypertrophy which is an adverse side effect caused by E2.

Example 3-5: Complete blood count

Complete blood count which reflects the condition and abnormality of the body was measured to find out abnormality in test animals caused by administrtion of agents. That is, to find out changes in hematopoiesis of test rats, measured were red blood cell(RBC) count, concentration of hemoglobin(Hb) and hematocrit(Ht) of blood samples obtained from the rats prior to operation and the rats 10 weeks after administrating agents following operation, and to find out changes in immune system such as inflammation and necrosis of tissues, measured were white blood cell count, lymphocyte count, monocyte count, and granulocyte count(see: Table 7).

Table 7: Changes in Complete blood count depending on drug administration

	Operation	Control group	Sham group	E2-treated group	Quercetin-treated group	Genistein-treated group
Red blood cell(RBC) count ($\times 10^6$ cells/μl)	before	7.36±0.11	7.19±0.11	7.33±0.13	7.29±0.15	7.32±0.13
	after	7.08±0.09	6.75±0.24	6.97±0.14	7.13±0.15	7.17±0.13
Concentration of hemoglobin(Hb) (g/dl)	before	16.09±0.21	15.75±0.20	15.86±0.24	16.00±0.30	15.82±0.27
	after	14.58±0.20**	14.09±0.48**	14.34±0.29**	14.84±0.22*	14.70±0.22**
Hematocrit(Ht) (%)	before	43.34±0.48	43.09±0.61	43.11±0.55	43.62±0.83	42.76±0.65
	after	39.48±0.60**	38.39±1.24**	38.86±0.72**	41.10±0.68*	40.66±0.56*
White blood cell count ($\times 10^3$ cells/μl)	before	26.13±4.63	25.61±3.64	23.14±1.50	20.28±3.77	27.30±4.85
	after	21.66±2.89	12.74±2.88*	13.26±0.97**	18.50±7.60	21.50±2.53
Lymphocyte count ($\times 10^3$cells/μl)	before	22.14±4.49	18.04±2.38	17.80±1.72	16.78±3.52	19.68±4.52
	after	21.20±9.00	10.20±2.88	10.23±0.96**	15.00±7.71	15.25±3.21
Monocyte count ($\times 10^3$cells/μl)	before	1.02±0.18	0.73±0.17	1.44±0.29	0.65±0.07	0.77±0.09
	after	1.10±0.21	0.95±0.14	1.02±0.24	1.00±0.20	0.80±0.19
Granulocyte count ($\times 10^3$cells/μl)	before	2.99±0.44	2.83±0.39	3.67±0.40	2.80±0.30	2.23±0.10
	after	2.52±0.21	1.93±0.26	1.99±0.25**	2.43±0.12	2.38±0.37

*: $p<0.05$,

**: $p<0.01$

As shown in Table 7, RBC count did not show any changes before and after operation in all groups, and concentration of hemoglobin and hematocrit were decreased after operation in all groups. White blood cell count did not show any changes before and after operation in quercetin or genistein treated groups, but decreased in Sham group and E2 group after operation. Also, lymphocyte

and granulocyte count showed rapid decrease in E2 group only, and momocyte count was stayed same in entire groups. Thus, quercetin was found to be a safe agent not disturbing hematopoiesis and immune system of the body.

Example 3-6: Biochemical changes of plasma by quercetin

Since blood reflects the condition of body, safety of quercetin in the body was evaluated by measuring biochemical parameters: that is, blood samples were obtained from the rat prior to operation, one week after operation, and 10 weeks after operation, and measured were levels of alkaline phosphatase(ALP), calcium, inorganic phosphate, blood urea nitrogen(BUN), creatinin, total cholesterol, HDL-cholesterol and LDL-cholesterol(see: Table 8).

Table 8: Changes in biochemical parameters in plasma depending on drug administration

	Operation	Control group	Sham group	E2-treated group	Quercetin-treated group	Genistein-treated group
Concentration of ALP (U/dL)	before	262.75±23.31	245.59±22.05	196.01±28.34	232.83±20.27	208.86±19.72
	1 week after	265.75±22.78	215.18±20.22	195.24±27.87	226.67±23.20	212.10±17.92
	10 weeks after	198.31±14.64	135.09±18.64$^{\#\#\$}$	123.99±22.18	156.42±13.08	127.14±9.95$^{\#\#\$\$}$
Concentration of calcium (mg/dL)	before	10.48±0.43	10.57±0.55	10.86±0.40	10.73±0.48	10.61±0.49
	1 week after	9.98±0.34	10.35±0.17	10.03±0.18	8.37±0.24$^{**\#}$	8.97±0.29$^{\#}$
	10 weeks after	10.83±0.16	11.79±0.23$^{*\$}$	11.20±0.16$^{\$}$	10.26±0.19$^{\$}$	10.44±0.22$^{\$}$
Concentration of inorganic phosphate (mg/dL)	before	6.52±0.39	6.87±0.62	6.90±0.52	6.79±0.66	7.18±0.48
	1 week after	6.27±0.31	6.59±0.20	6.13±0.12	6.21±0.18	6.47±0.16
	10 weeks after	4.95±0.41$^{\#\#}$	6.09±0.47	5.51±0.45	5.73±0.58	5.62±0.25$^{\#}$

Concentration of blood urea nitrogen(BUN) (mg/dL)	before	18.56±0.92	17.13±1.11	18.36±1.01	17.05±0.60	16.82±0.60
	1 week after	18.31±0.70	16.75±0.58	17.79±0.76	18.06±0.88	18.26±0.94
	10 weeks after	21.20±1.06	19.23±0.84	19.99±0.86	18.19±0.41	18.31±0.86
Concentration of creatinin (mg/dL)	before	0.54±0.05	0.56±0.06	0.55±0.05	0.57±0.05	0.51±0.04
	1 week after	0.54±0.05	0.62±0.04	0.57±0.03	0.59±0.01	0.64±0.02*
	10 weeks after	0.78±0.03 ##$$	0.80±0.03 ##	0.81±0.03 ##$$	0.82±0.04 ##$	0.82±0.04 ##$
Concentration of total cholesterol (mg/dL)	before	72.66±5.00	79.67±1.73	76.79±2.80	77.55±5.13	85.51±5.45
	1 week after	93.32±4.75 #	79.75±2.46	95.53±4.17	85.84±3.82	91.56±3.65
	10 weeks after	120.44±5.21 ##$$	88.60±4.87 **#	115.05±5.75 ##$	107.73±2.24 ##	121.07±6.53 ##
Concentration of HDL-cholesterol (mg/dL)	before	53.78±2.77	52.33±2.61	52.30±2.01	53.38±3.14	61.12±3.57
	1 week after	46.20±0.62	41.69±1.47	49.03±3.37	42.49±4.85	35.26±1.92 # #
	10 weeks after	29.60±2.63 ##$$	22.32±2.49 ##$$	24.94±2.72 ##$$	25.13±2.78 ##	29.27±1.98 # #
Concentration of LDL-cholesterol (mg/dL)	before	18.88±3.15	26.63±3.04	24.49±1.63	24.17±3.13	24.39±3.63
	1 week after	42.80±6.41 ##	36.30±0.63	40.50±6.17	40.85±4.88	60.47±7.04 # #
	10 weeks after	90.84±4.27 ##$$	69.29±3.05 ##$$	88.33±4.74 ##$$	82.60±4.85 ##$$	91.80±6.57 # #$$

*: p<0.05, **: p<0.01, compared with control group

#: p<0.05, ##: p<0.01, compared with before operation

$: p<0.05, $$: p<0.01, compared with 1 week after operation

As shown in Table 8, ALP activity which is directly related to bone metabolism showed tendency of decrease with aging in entire groups, especially, in Sham group and genistein treated group, the rats of 10 weeks after operation showed significant decrease of ALP activity and no change in calcium concentration compare to the rats prior to operation and one week after operation. And, the level of inorganic phosphate remarkably decreased in the

rats of 10 weeks after operation compare to the rats prior to operation in control group and genistein treated group.

While the level of blood urea nitrogen which is related to the protein metabolism and muscle volume was maintained at a proper level in entire groups, the level of creatinin increased in entire groups.

The level of total cholesterol which is known to increase in postmenopause women increased in entire groups, although increase in Sham group was relatively low. While the level of HDL-cholesterol decreased with time in entire groups, the level of LDL-cholesterol increased with time, which were found in normal Sham group as well as ovariectomized groups.

Thus, the quercetin of the invention was found to be an effective therapeutic and preventive agent for osteoporosis.

Example 4: The formulation of the quercetin preparation

Example 4-1: Syrup

The syrup formulation containing 2%(w/v) quercetin, its derivatives or pharmaceutically acceptable salts thereof was prepared as follows: quercetin hydrochloride, saccharine and sugar were dissolved in 80g of warm water, cooled down, and then mixed with a solution containing glycerin, saccharine, aromatics, ethanol, sorbic acid and distilled water. Water was added to the mixture prepared above to give 100ml of syrup formulation of quercetin, whose components are as follows:

quercetin hydrochloride ····· 2g
saccharine ····· 0.8g
sugar ·····25.4g
glycerin ····· 8.0g
aromatics ·····0.04g
ethanol ····· 4.0g

sorbic acid ····· 0.4g
distilled water ····· a proper quantity

Example 4-2: Tablet

The tablet containing quercetin, its derivatives or pharmaceutically acceptable salts thereof was prepared as follows: 250g of flavonoid derivative of quercetin · hydrochloride was mixed with 175.9g of lactose, 180g of potato starch, and 32g of colloidal silicate, and then 10%(w/v) gelatin solution was added. After pulverization, the mixture was passed through a 14-mesh sieve, dried, and mixed with 160g of potato starch, 50g of talc, and 5g of magnesium stearate to give tablets, whose components are as follows:

flavonoid derivative of quercetin · hydrochloride ·250g
lactose ····· 175.9g
potato starch ····· 180g
colloidal silicate ····· 32g
10%(w/v) gelatin solution ····· a proper quantity
potato starch ····· 160g
talc ····· 50g
magnesium stearate ····· 5g

Example 4-3: Injection

One gram of flavonoid derivative of quercetin · hydrochloride, 0.6g NaCl, and 0.1g of ascorbic acid were dissolved in distilled water to give a final volume of 100ml, and then the solution was put into a vial, which was sterilized by heating at 100°C for 30 minutes to give the injection. The components of the said injection are as follows:

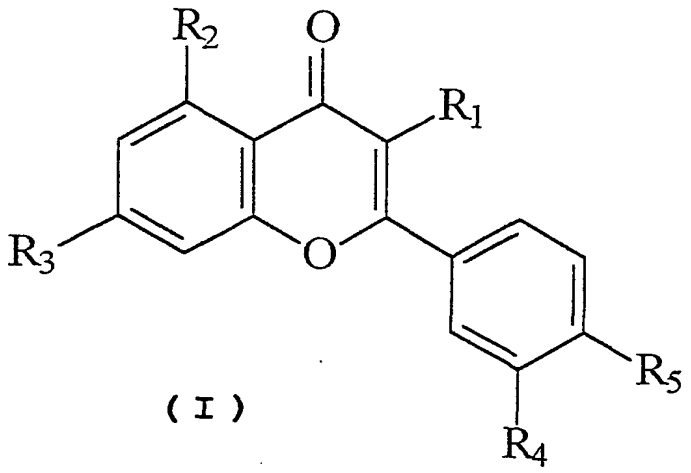
flavonoid derivative of quercetin · hydrochloride ····1g
NaCl ····· 0.6g

ascorbic acid ····· 0.1g
distilled water ····· a proper quantity

As clearly illustrated and demonstrated above, the present invention provides a therapeutic agent for osteoporosis comprising an active ingredient of quercetin derivatives which effectively stimulate osteoblast proliferation and inhibit osteoclast proliferation. The quercetin derivatives of the invention can be practically applied for the treatment and prevention of osteoporosis, since they effectively inhibit osteoclast proliferation and stimulate osteoblast proliferation more than conventional therapeutic agents for osteoporosis, and increase trabecular bone area highly without changing hormone level in body and untoward effects on hematopoietic function and immune system.

WHAT IS CLAIMED IS:

1. A therapeutic agent for osteoporosis comprising an active ingredient of quercetin derivatives represented by the following general formula(I) and a pharmaceutically acceptable carrier:



(I)

wherein,

R_1 is gentiotriose, glucopyranose, O-arabinofuranose, O-diglucopyranose, O-galactopyranose, O-galactoside-gallate, O-gentiobiose, O-glucopyranose, O-glucuronide, O-neohesperidose, O-rhamnopyranose, O-rutinose, O-sophorose, O-xylopyranose, OCH_3, OH, rhamnogentiobiose, rhamnoglucose or sulfate;

R_2 is OH or O-glucopyranose;

R_3 is OCH_3, OH, O-glucopyranose, O-glucuronopyranose or glucopyranose;

R_4 is OCH_3 or OH; and,

R_5 is OCH_3, OH, O-glucopyranose or O-glucose.

2. The therapeutic agent for osteoporosis of claim 1, wherein the quercetin derivatives are compounds represented by general formula(I) whose R_2, R_3, R_4 and R_5 are -OH as followings: quercetin, avicularoside, guiajaverin, hyperoside, isohyperoside, isoquercitrin, multinoside A, multinoside A acetate, quercitrin, rutin, quercetin-3-O-

(2"-O-β-D-glucopyranosyl)-α-L-rhamnopyranoside, quercetin-3-O-(6"-O-galloyl)-glucopyranoside, quercetin-3-O-(6'"-O-p-coumaroyl-β-D-glucopyranosyl-(1-2)-α-L-rhamnopyranoside), quercetin-3-O-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl-(1-4)-α-L-rhamnopyranoside, quercetin-3-O-[2"-O-6'"-O-p-(7""-O-β-D-glucopyranosyl)coumaroyl-β-D-glucopyranosyl]-α-L-rhamnopyranoside, quercetin-3-O-[6'"-p-coumaroyl-β-D-glucopyranosyl-β-(1-4)-rhamnopyranoside], quercetin-3-O-[α-L-rhamnopyranosyl(1-2)-α-L-rhamnopyranosyl -(1-6)-β-D-glucopyranoside], quercetin-3-O-[α-rhamnopyranosyl(1-4)α-L-rhamnopyranosyl(1-6)β-D-galactopyranoside], quercetin-3-O-[α-rhamnopyranosyl-(1-2)]-[β-glucopyranosyl-(1-6)]-β-D-galactopyranoside, quercetin-3-O-[α-rhamnopyranosyl-(1-4)-α-rhamnopyranosyl-(1-6)-β-galactopyranoside], quercetin-3-O-α-L-rhamnopyranosyl-(1-2)-β-D-galactopyranoside, quercetin-3-O-β-D-diglucopyranoside, quercetin-3-O-β-D-galactoside-2"-gallate, quercetin-3-O-β-D-glucopyranoside -(1-6)-β-D-galactopyranoside, quercetin-3-O-β-D-glucopyranosyl-(1-3)-α-L-rhamnopyranosyl-(1-6)-β-D-galactopyranoside, quercetin-3-O-β-D-glucuronide, quercetin-3-O-β-D-xylopyranoside, quercetin-3-O-diglucospyranoside, quercetin-3-O-gentiobioside, quercetin-3-O-glucopyranosylgalactopyranoside, quercetin-3-O-neohesperidoside, quercetin-3-gentiotrioside, quercetin-3-methyl ether, quercetin-3-rhamnogentiobioside, quercetin-3-rhamnoglucoside, or quercetin-3-sulfate.

3. The therapeutic agent for osteoporosis of claim 1, wherein the quercetin derivatives are compounds represented by general formula(I) whose R_1 is -OH and three functional groups out of R_2, R_3, R_4 and R_5 are -OH as followings: isorhamnetin, quercimeritrin, rhamnetin, quercetin-5-O-β-D-glucopyranoside, quercetin-7-O-β-D-glucuronopyranoside or spireaoside.

4. The therapeutic agent for osteoporosis of claim 1,

wherein the quercetin derivatives are compounds represented by general formula(I) whose three functional groups out of R_1, R_2, R_3, R_4 and R_5 are -OH as followings: rhamnazin, quercetin-3',4'-di-methyl ether, quercetin-3,3'-dimethyl ether, quercetin-3,7-dimethyl ether, quercetin-3-O-[2"-O-(6'"-O-p-coumaroyl)-β-D-glucopyranosyl]-α-L-rhamnopyranosyl-7-O-β-D-glucopyranoside, quercetin-3-O-[2"-O-6'"-O-p-(7""-O-β-D-glucopyranosyl)coumaroyl-β-D-glucopyranosyl]-α-L-rhamnopyranoside-7-O-β-D-glucopyranoside, quercetin-3-O-rutinoside-7-O-β-D-glucopyranoside, quercetin-3-O-α-L-arabinopyranosyl-7-O-β-D-glucopyranoside, quercetin-7-O-β-D-glucopyranoside-3-O-sophoroside, quercetin-3-O-galactopyranosyl-7-O-diglucopyranoside, quercetin-3-O-glucopyranosyl-7-diglucopyranoside, quercetin-3,7-diglucopyranoside, quercetin-3-gentiobiosyl-7-glucopyranoside or quercetin-3,4'-di-O-β-D-glucopyranoside.

5. The therapeutic agent for osteoporosis of claim 1, wherein the quercetin derivative is quercetin-3,4',7-trimethyl ether or quercetin-3,3',4',7-tetramethyl ether.

6. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is selected from the group consisting of polyvinylpyrolidone and hydroxypropylcellulose.

7. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is a disintegrating agent selected from the group consisting of calcium carboxymethylcellulose and sodium glycolate starch.

8. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is a diluting agent selected from the group consisting of corn starch, lactose, soybean oil, crystalline cellulose and mannitol.

9. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is a lubricating agent selected from the group consisting of magnesium stearate and talc.

10. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is a sweetener selected from the group consisting of sucrose, fructose, sorbitol and aspartame.

11. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is a stabilizing agent selected from the group consisting of sodium carboxymethylcellulose, α- or β-cyclodextrin, vitamin C, citric acid and white wax.

12. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is a preservative selected from the group consisting of paraoxymethylbenzoate, paraoxypropylbenzoate and sodium benzoate.

13. The therapeutic agent for osteoporosis of claim 1, wherein the pharmaceutically acceptable carrier is an aromatic selected from the group consisting of ethylvanillin, masking flavor, flavonomenthol and herb flavor.

14. The therapeutic agent for osteoporosis of claim 1, wherein the therapeutic agent is a pharmaceutical formulation for oral or parenteral administration selected from the group consisting of tablets, capsules, soft capsules, liquids, ointments, pills, powders, suspensions, emulsions, syrups, suppositories and injections.

15. The therapeutic agent for osteoporosis of claim 1 which further comprises calcium or vitamin D_3.

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/353

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)

IPC7: C07D; A61K

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)

CASLINK; ESPACENET

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 63-156720 A (KISSEI CO.) 29. 06. 88, see the whole document, (Family; none)	1-15
A	WO 95/03293 A (CHINOIN LTD.) 02. 02. 95, see the whole document	1-15
A	JP 60-048924 A (TAKETA LTD.) 16. 03. 85, see the whole document	1-15
A	US 6,040,333 A (SHERRY D.) 21. 03.00, see the whole document, (Family; none)	1-15
A	Fiorelli, G. et al., "Estrogen synthesis in human colon cancer epithelial cells", In; J. Steroid Biochem. Mol. Biol., 1999, 71(5-6), 223-230.	1-15

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevence

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 JUNE 2001 (27.06.2001)	29 JUNE 2001 (29.06.2001)

Name and mailing address of the ISA/KR	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR01/00368

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